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14. ABSTRACT
In year 3, we recruited 5 more qualified undergraduate students from Lincoln University and Delaware State University (DSU) to participate in our prostate cancer research and health disparity training program. All students did 10 week, hands on research in laboratories at the University of Delaware (UD) capped by poster presentations in our annual undergraduate research symposium. Faculty from UD gave research talks on prostate cancer and health disparities at Lincoln and DSU during the semester. Research efforts through the school year at the HBCU were difficult to continue and required a commitment from the students to return to UD in order to continue. This is changing as more research laboratories come online at Lincoln and DSU but this is a very slow process. Overall, we have recruited 15 undergraduate minorities into our training program in compliance with our aims and 14 have finished. We have placed students in laboratories in Biology, Chemistry/Biochemistry and Physics at UD. The summer enrichment programs provided by HHMI and our own health disparity discussions are very popular. A large percentage of trainees applied to graduate schools.

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Introduction:
Due to the extremely low levels of minority faculty and graduate students in the sciences, the DoD Majority Institution (MI) /Historically Black College and University (HBCU) program was intended to foster and promote the interest of underrepresented minority students in basic sciences by offering students from HBCUs research opportunities at sponsoring MIs. In Delaware, student from Delaware State University (DSU) and Lincoln University (LU) have been recruited to perform summer research in funded prostate cancer laboratories at the University of Delaware (UD). Recruitment efforts have focused on students who were interested in earning advanced degrees. Our aims were to offer 1) a 10-week summer research program to five qualified minority students, 2) a summer enrichment program and 3) activities and extended research at the participating HBCUs during the following academic year. Our program has: 1) successfully recruited highly qualified minority students from both DSU and LU, 2) successfully guided 14 of 15 students, who entered the program, to the completion of the program's requirements, 3) offered seminars at the minority institutions that were given by four UD faculty, 4) instituted round table discussions about health disparities into our summer enrichment program and 5) taken students to national and local scientific meetings.

Body:
In compliance with Aim 1 for year three of our program, and upon the recommendation of the faculty campus coordinators at Delaware State (Dr. Cynthia van Golen) and Lincoln University (Dr. Susan Safford), three students from LU (**Kiamesha Castle and Mashariki Jenkins-Kabaila**) and two students from DSU (**Wachen Peters, Alfayo Michira, and Danielle Daniels,**) were chosen for admission into the University of Delaware's training program in Prostate Cancer. Each student chosen was recognized by the campus coordinator as being academically excellent (3.0 grade point average or above) and motivated to do research. Students in the program participated through "hands-on" research in their designated mentors' laboratories for 10 weeks during the summer. Danielle Daniels was mentored by Dr Ken van Golen and Carlton Cooper. Kiamesha Castle was mentored by Drs. R. Duncan and Robert Sikes. Mashariki Jenkins was mentored by Dr. van Golen and Wachen Peters was mentored by Dr. Sikes. Alfayo Michira was mentored by Dr. Koh in Chemistry and Biochemistry.

Overall, our research program has matriculated 15 students over three years with 14 completing the program as outlined in Table 1. There are only 13 names in the table since Mashariki Jenkins-Kabaila spent two summers in the program. Our program had to replace Charles Wilson in year 2 due to health issues. He was replaced by Cynthia van Golen, Ph.D. the program really did not suffer from the switch as student recruitment and interest had already been established.

Table 1. DoD HBCU/MI Summer Research Scholars

Name	**Program Year**	**Research Mentor**	**Presentations/Pubs (Yes or No)/(Yes or No)**
Candice Johnson	2006	Koh & Wommack	Yes/no
Erin Stallings	2006	DeLeon	Yes/no
Lauretta Ovadje	2006	Sikes	Yes/in preparation
Noella Zony	2006	Cooper	Yes/no
Renee Dixon	2006	Sikes	Yes/no

Adaire T. Heady	2007	Sikes & Hadjipanayis	Yes/no
Brenda Mogere	2007	Sikes	Yes/
Mashariki Jenkins-Kabaila	2007, 2008	Van Golen	Yes/
Osemeke Edobor	2007	Sikes, Van Golen & Cooper	Yes/
Alfayo Michira	2008	Koh	Yes/
Danielle A. Daniels	2008	Van Golen & Cooper	
Kiamesha N. Castle	2008	Sikes & Duncan	Yes/no
Wachen Peters	2008	Sikes	Yes/in preparation

In compliance with Aim 2, students attended weekly seminars related to research http://www.udel.edu/chem/white/HHMI3/Summer08/S08enrichment.html. In addition our students attended roundtable discussion sessions on the topic of *Healthcare Disparities*. These discussion sessions were not proposed in the grant but were quite successful and popular as judged by a survey that students were required to fill out. Prior to each session students were assigned to read both popular and scientific literature regarding the socio-economic or medical causes of healthcare bias. In our first session we focused on Socioeconomic factors. For our second session we focused on medical factors and race-based medicine. The conversation was really quite stimulating.

Over the three year period, this luncheon series on Healthcare Disparities attracted about 20-25 students per session. These students came from the HHMI and NUCLEUS summer programs and provided a nice blended environment within which to discuss Health Disparities. Being moderated sessions, all discussion was kept cordial and respectful. Several students in my laboratory (Sikes) expressed a great deal of enthusiasm for these luncheons and frequently continued the discussion long afterwards. In addition to the PI and Co-PI, we had faculty participation from biology, physics, chemistry and psychology. The HHMI and NUCLEUS program coordinator also was a significant contributor to the discussion and ensured the success of the luncheons by following up with students to ensure their participation.

In compliance with Aim 3, four faculty lectures were given during the academic year. Kenneth van Golen gave two lectures, one at DSU and one at Lincoln, and Carlton Cooper one at LU and one at DSU. A conference on Cancer Disparities was hosted by Lincoln University and Fox Chase cancer Center where Dr. Sikes was an invited Speaker.

We produced 4 faculty seminars on Health disparities AND cancer each of the three years of this program. Topics included prostate cancer, obesity and cancer, diet and cancer, breast cancer, and pancreatic cancer among others. Seminars were well attended and were given by Drs. Sikes, Usher, Cooper and van Golen.

Key Research Accomplishments:
The students in this program were expected to make significant progress in research over a 10 week period. Much of this time was spent instructing them in laboratory procedures that included basic liquid handling, safety, and use of technology and equipment required. Despite this, the amount of publishable data that each student collected during this short time is amazing. Additionally, students were instructed to journal their research experience to enhance their level of comfort of communicating what skills and techniques they learned as well as understanding the research project. At the end of the summer program, each student presented the results of their research at the University's undergraduate research symposium. The symposium was modeled after the Experimental Biology meeting, where posters and talks occurred simultaneously and where there was a plenary lecture by a Howard Hughes Medical Institute investigator http://www.udel.edu/chem/white/HHMI3/Summer08/S08enrichment.html. The summer symposium taught the students how to communicate their findings to an audience of faculty, postdoctoral students and graduate students through poster presentation. Criticisms at this symposium led to changes in their posters which were presented at national meetings.

This was key in providing students feedback and a level of comfort that was obvious at their presentations of results at the IMPaCT meetings held in Atlanta in September 2007. In preparation for national meetings such as ABRCMS, pre-conference meetings were held to coach students on improving their presentation skills as well as networking ability and conference etiquette. Mentors and the NUCLEUS coordinator spent hours coaching students on poster presentations and answering questions about their work.

Reportable Outcomes:
Abstracts and Poster Presentations
Students were required to present their posters at the UD Undergraduate summer research symposium. This symposium is attended by over 300 students and faculty annually. It includes a nationally recognized speaker and oral talks by selected abstracts. The link for 2008 can be found at http://www.udel.edu/chem/white/HHMI3/Symp08/SympParticipants08.html where the DoD summer scholars can be located by name.

Abstracts for students presenting in year 3 of the program are listed below:

1. **Evaluating the Molecuar Mechanisms of Anti-Androgen Resistance**
Alfayo Michira, **John T.Koh**, and Kathy Miller
Department of Chemistry and Biochemistry

Androgen-dependent prostate cancer can be treated with anti-androgens, however, in time many cancers become hormone refractory and no longer respond to the drugs. Mutations on the androgen receptor (AR) have been identified to play a major role in anti-androgen resistance. In some cases anti-androgens activate the androgen receptor stimulating the growth of the cancer, which has proved difficult to treat.The Koh group, is involved in designing compounds that target mutant hormone receptors. A new anti-androgen PLM6 was found not to form resistant clones in culture. PLM6 may evade resistance mechanisms or may simply be more toxic. By growing the LNCaP cell line, we studied their proliferation in the presence of different concentrations of bicalutamide

and PLM6 over a short-term growth period. Two growth assays (Cyquant and titer blue) are used to evaluate their growth. We also did site-directed mutagenesis where we created some AR mutations that were found in resistant cells. The findings of this study indicate that PLM6 is toxic rather that evading the AR resistance mechanisms. Funded by DoD grant.

2. **Potential Role of Adenosine Triphosphate (ATP) in Prostate Cancer Metastasis to Bone**

Kiamesha N. Castle, Christine Maguire, Patricia Jones, **Robert Sikes** and **Randall L. Duncan**
Department of Biological Sciences
*also presented at TriBeta Biological Honors Society Meeting March 14, 2009

Prostate Cancer (PCa) is the second most common type of cancer among men and results from the uncontrolled growth of epithelial cells that line the ducts of the prostate gland. The cause of death in these men is rarely attributed to the original tumor, but rather the metastasis of the cancer to its primary targets, bone and lymph. Our lab has shown that bone cells release ATP in response to a number of stimuli. ATP then signals surrounding cells through purinergic receptor activation to induce bone formation. We postulate that this release may play a role in the affinity of PCa cells to bone and that more metastatic cells release greater amounts of ATP. To test this hypothesis, we examined levels of ATP released from cells of the LNCaP model; LNCaP, C4-2 and C4-2B cells. These cells were developed to become increasingly metastatic. LNCaP cells representing the least metastatic and C4-2B cells being the most aggressive. Our lab has demonstrated that the release of ATP is mediated through entry of calcium into the cell. We treated the three cell lines with ionomycin, a calcium ionophore. We found that the C4-2B cells released the greatest amount of ATP compared to untreated control cells and that LNCaP cells released the least. These studies suggest that more metastatic cells release large amounts of ATP to stimulate cell migration and invasion of PCa cells into bone. Future studies will focus on the mechanism of ATP release from the metastatic cells and the effector response of these cells to ATP. (Funded by Department of Defense PCRP-W81XWH-06-1-0244 and NIH/NIAMS R01 AR051901)

3. **The effect of IGF-1 and RhoC GTPase on Prostate Cancer Cell adhesion to Bone Marrow Endothelial Cells**

Danielle A. Daniels[1], Cara W. Dubyk, **Kenneth van Golen**, and **Carlton Cooper**
Department of Biological Sciences, University of Delaware; [1]Delaware State University

RhoC is a small signaling protein, more specifically a monomeric GTPase, and is a member of the Ras sub family. Rho proteins are involved in multiple cellular processes, such as cell division, intracellular trafficking, and the organization of cytoskeletal components. The RhoC protein has been shown to be directly involved in cancer cell motility and invasion. Breast cancer, inflammatory breast cancer, and pancreatic cancer are among a few of the cancers in which RhoC is known to play an active role. Insulin-like Growth Factor (IGF-1) plays a significant role in cell growth regulation and

development, as well as cellular DNA synthesis. This experiment uses three different cell lines to determine their ability to adhere to Bone marrow endothelial cells (BMEC) in the presence of IGF-1. The three types of PC-3 cell lines used were parental PC-3, PC-3's containing a dominant negative RhoC (dnRhoC), and a control PC-3 vector Lac Z. These cells were treated with IGF-1 for varying time points ranging from 5 to 30 minutes, and their adhesion to BMEC cells was measured by performing an adhesion assay. The hypothesis of this experiment is that IGF-1 regulates PC-3 adhesion through RhoC GTPase activity. Prostate cancer cell adhesion to Bone marrow endothelial cells is a critical step in invasion and metastasis to bone, which is a major clinical concern. Funding for this project has been provided by the Department of Defense.

4. **The role of purinergic signaling in an isogenic progression of prostate cancer cells**

Wachen Peters, Christine Maguire, Adam Aguair, and **Robert A. Sikes**
Department of Biological Sciences
*also presented at TriBeta Biological Honors Society Meeting March 14, 2009

Background: Purinergic signaling stimulates many biological processes such as cell proliferation, differentiation, and apoptosis. Two classes of purinergic receptors, GPCR and S/T kinase, have been identified that bind ATP and other nucleotides as ligands. ATP has an antitumor effect on cancer in vivo. Thus clinical trials are being carried out to determine that ATP can be used as a therapeutic agent for cancer. Here we looked at the effect of ATP on an isogenic progression series of prostate cancer (PCa) cell lines (LNCaP, C4 2, and C4 2B4). We hypothesize that ATP stimulates neuroendocrine differentiation (NED) and growth in PCa cells. NED occurs with relatively high frequency in PCa and is correlated directly with poor prognosis. Methods: ATP, 0.001-1000µM, was added in log10 increments to PCa cell lines in vitro. Morphology was examined by photomicroscopy; cell number was determined using crystal violet staining; and, migration was examined using scratch assays. Results: ATP increased the growth of PCa cells over vehicle alone (LNCaP=2.7X, C4-2= 1.7X, C4-2B4= 1.4X) with peak stimulation occurring at 1nM ATP. Morphology was largely unchanged in response to ATP. Scratch assays are currently in progress. Conclusions: Growth response to exogenous ATP decreases as PCa cells become more androgen insensitive and metastatic. NED is not apparent morphologically but biochemical analysis is required to confirm this result. Funding by DoD PCRP-W81XWH-06-1-0244

5. The Roles of RhoG, Rac1, and Rac3 GTPase in PC-3 Human Prostate Cancer Tumor Cell Diapedesis

Mashariki Jenkins-Kabaila, Cara Dubyk, Moumita Chatterjee, and **Kenneth Van Golen**
Laboratory of Cytoskeletal Physiology, The Department of Biological Sciences, Center for Translational Cancer Research,

Based on previous research, the downregulation of the RhoC GTPase in PC-3 human prostate cancer cells derived from bone metastasis leads to increased and sustained levels of Rac GTPase activity. It has been shown that the Rac GTPases are involved in prostate cancer cell migration and invasion particularly through bone marrow endothelial cells. There are 3 isoforms of Rac

and a homologous protein, RhoG, that has been implicated in the activation of Rac1. In the current study, we examine the levels of expression, activation, and phenotypic effects of Rac1, Rac3 and RhoG GTPases. The relative and quantitative levels of Rac1, Rac3, and RhoG were compared in PC-3 cells, C3 exotransferase (an inhibitor of RhoC GTPase) treated PC-3 cells and siRNA treated cells. A tumor cell diapedesis assay was done across a monolayer of bone marrow endothelial cells after the siRNA treatment of Rac1, Rac3, or RhoG to determine the individual contributions of each GTPase to a cell's invasive capability. RhoG, Rac1, and Rac3 ability to undergo diapedsis was tested. In the future a GLISA experiment will be done on siRNA treated PC-3 cells. We will determine the phenotypic and physiological effects of Rac1, Rac3, and RhoG more closely. As well, we plan to calculate changes in morphology by determining the contribution of each GTPase to the formation of lamelipodia.

Conclusions:

For this last year we find that 14/15 students generated publishable data in the form of multiple abstracts and most of the 14 presented their results at national meetings. When the science programs at the HBCU and meetings are totaled, approximately 30 abstracts can be accredited to DoD scholars in our program. A few papers are in preparation as listed in Table 1. Over three years, we have made changes required to increase communication between the faculty involved in research at the HBCUs during the academic year. Coordination of research projects consistent with the capabilities of the HBCU definitely is required to minimize student commuting during the academic year and maximize research productivity at the HBCUs. Despite this, research during the year at the HBCU was stopped for all intensive purposes after year 2. Finally, it appears that we have increased the number of minority students applying to graduate schools although tracking the final outcome has been difficult due to lost contact with students following graduation.

References:

Not Applicable to date.

Appendices:

None-See abstracts for year 3 reprinted above.